

Basal lignified star-shaped cavity in oil palm (*Elaeis guineensis* Jacq.): early development in nursery and consequences for BSR control¹

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ABSTRACT

The existence of a high lignified star-shaped cavity at the base of the bole, or more precisely at the root – bole interface, has been clearly identified for the first time. This cavity appears to be a perfect culture chamber for Ganoderma development. Random observations of the initial stage of Basal Stem Rot infection in young palms showed the development of infectious stroma-like structures inside the cavity prior to colonisation of the bole. The existence of a lignified scar in 8-month-old seedlings was revealed by initial observation. The scar then developed further inside the star-shaped cavity (Breton et al, 2009a; Breton et al, 2009b).

Specific observation during early growth stages in the nursery revealed the appearance of a very tiny scar after 5 months on a few seedlings. The number of affected seedlings increased rapidly to reach nearly 100% after 9 months. The scar grew quickly in all directions and the resulting cavity could reach 50% of bole diameter, 13 mm wide and 8 mm deep at this stage. The star-shaped cavities have 2 to 5 branches.

After 6 to 7 months in the nursery, once the star-shaped cavity was extended enough, the development of mycelium (not identified) and / or presence of tiny arthropods such as aphids, Chilopodae larva and small ants were frequently observed. These results demonstrate the role of this star-shaped cavity as a perfect culture chamber for fungus and a possible refuge for pests, predators or disease vectors at very early stages of oil palm development.

This star-shaped cavity definitely appears to be an interesting target for preventive action against Basal Stem Rot, i.e. Biocontrol by antagonistic, long-lasting fungicide, etc.

Key words: *Elaeis guineensis*, palm oil, lignified star-shaped cavity, nursery, basal stem rot

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INTRODUCTION

Ganoderma boninense, which causes a Basal Stem Rot (BSR) on *Elaeis guineensis* Jacq. is the most dangerous soil-borne fungus. It is wide spread across oil palm estates in Southeast – Asia. The disease appears to be increasingly economically important with the accumulation of oil palm growing in the same place. Fields planted with susceptible material could be rapidly devastated and the remaining density could be reduced to eighty trees per ha after 18 to 20 years of production.

The presence of high melanised mycelia or stroma-like structure (SLS) is necessary for infection. SLS require specific conditions to develop: hard substrate, dark environment, humidity and low competition (Breton *et al*, 2005a; Breton *et al*, 2005b).

Extensive observations on oil palms at all stages and on all continents have revealed the existence of a lignified star- shaped cavity at the root bole interface. SLS have been detected on the outer section of this star-shaped cavity (Breton *et al*, 2009a). Random observation of the initial stage of infection on young palms showed that SLS development occurs before penetration and colonisation of the palm bole (Breton *et al*, 2009b).

The star-shaped cavity displays all the prerequisite qualities to be a perfect culture chamber for *Ganoderma boninense* SLS: hard substrate, dark environment, high humidity and low competition. A scar, a future star-shaped cavity has been observed on the root – bole interface of eight and eighteen-month-old seedlings (Breton *et al*, 2009a; Breton *et al*, 2009b).

In order to have a clearer understanding of cavity development, an experiment was carried out in the nursery at the Aek Loba Estate. The aim was to detect the possible appearance of the scar as early as possible and, if possible, understand how the scar was developing inside the cavity.

EXPERIMENT

MATERIALS

400 five-month-old seedlings originating from one D Dabou Deli x T la Mé cross transferred in the nursery in early March 2009.

Nursery bag:	Socfindo standard
Nursery spacing:	Socfindo standard
Water supply:	Socfindo standard
Fertilisation:	Socfindo standard

METHODS

20 seedlings were sacrificed each month. They were taken following their rank in the nursery without choice. The nursery bags were opened with a knife and the soil carefully removed in order to keep all the roots in their original state. The seedlings were transferred to the nursery office.

At the office, all the primary roots were cut up to two centimetres from the bole. They were, then, examined and divided into four categories: new, normal, injured and rotten.

- New roots: freshly emitted roots, white, turgescient and without any secondary root emission
- Normal roots: roots with normal shape
- Injured roots: roots bearing within their first 30 centimetres one or more re-growing sections or rotten section not joining the bole
- Rotten root: root that is rotten up to the bole

Then, with a very sharp large knife, the remaining root bases were cut off until the bole – root interface was reached. This operation was very tricky, because the thickness of this interface was a very tiny target at the beginning.

After cleaning, the bole – root interface was examined and all the possible observations were recorded such as the existence or not of any early signs of a scar or cavity, mycelium, fungus, or insects.

After initial observations, we added characterisation of scar morphology, then cavity measurements (extension, width and depth) and, at least for the last observation, bole diameter. All measurements were taken with a calliper square.

Eight dissections were carried out from 20/04/2009 to 27/01/2010 (Table 1). Pictures were taken to characterise all significant steps.

RESULTS

The first series of data concerned root emissions (Table 2). After 1 month in the nursery, around 60% of the roots were injured by the transfer process. After that, the number of injured roots decreased regularly, probably through the natural process of decay and replacement. On average, the number of new roots rose from 2 at 1 month to 4 – 5 between 8 and 11 months old. In parallel, the number of rotten root bases detected on the bole increased slowly with a peak in the 7th month. The total number of active primary roots was multiplied by 4 over the 10 months of observation.

The first suspicion of a scar appeared during the second series of observations on 05/05/2009, i.e. after 1.7 months in the main nursery. Table 3 summarises scar and cavity presence for each dissection series. Between second and third dissections, around 35 to 40% of dissected seedlings showed suspicious markings at the root – bole interface. Cavities were clearly identified after 5 months in the nursery on 55% of the seedlings. All the seedlings displayed a cavity after 8 months in the main nursery.

Cavities differed in appearance: an analysis of their morphology can be founded in Table 4. Basically, 4 morphological types were identified (see picture gallery):

- S2: lengthened cavity with 2 extremities like irregular distaff
- S3: star-shaped cavity with 3 irregular branches
- S4: star-shaped cavity with 4 irregular branches
- S5: star-shaped cavity with 5 irregular branches

Width and depth of cavities observed during the fourth dissection (5.4 months in the main nursery) measured less than 2 mm. These cavities then grew significantly in all directions. Table 5 summarizes development of the cavity at the root – bole interface. At 6.8 month-old,

the cavity reached already 15 mm in length and 3 mm in width on average. Three months later, the cavity size had doubled and amounted to 40% of the bole diameter.

DISCUSSION

Very early occurrence of a scar or cavity was not that easy to identify because we did not know what we were looking for. Figure 1 shows the appearance of normal root – bole interface. Successive root sections could be clearly identified. At some places, lignified spots indicated the position of rotten roots (see arrow).

From time to time, rotten root bases appeared on the bole – root interface. This was very clear on 1 to 3-month-old seedlings. Rotten tissues invaded the area around the root, but generally not its central cylinder (Fig 2). This figure did not generate a scar or lignified cavity at this stage.

Early development of the cavity

The very earliest cases we were able to recognise were small root bases, narrowing at the middle of the bole – root interface, bearing one or two tiny cracks, 1 to 2 mm in length, starting to divide its section into two parts. Such crack development appeared, initially, external to the root (Fig 3) then grew through the root (Fig 4).

Very soon afterwards, a scar is developed like an irregular meandering line at the bole – root interface (Fig 5). After a few weeks, the width and the depth of the scar increased allowing development of the first cavity stages. Well developed cavities were always 4 to 6 times longer than the width. The depth also appeared to be always slightly smaller than the cavity width.

As mentioned in the results, this cavity could develop 2 to 5 irregular branches. The picture gallery in the annex shows the morphology of such cavities.

At some bole – root interfaces (Fig 6), we saw appearance of rotten tissues at the narrowing tip of a star branch. Rotten tissues were a pale brownish-yellow colour and really seemed to be extending the cavity from a root base. A vertical section along the cavity length (Fig 7) showed already lignified tissues limiting the cavity deeper in the bole and around the root base, the early beginnings of scarring.

Observation carried out earlier by the authors indicated that SSC development also depended on the genetic background, the origin of the planting material, or growth / stress conditions, as shown by cavity extension at the bole – root interface of adult palms in Indonesia, Africa or South America (Breton *et al*, 2009a). More studies need to be undertaken on that subject in the future.

Cavity colonisation

At very early stages, the cavity was refilled with rotten and dry tissues, as shown in figure 7. But very quickly, as can be seen from the picture gallery, the cavity developed free space at the bole – root interface

Very early, this cavity was colonised by tiny animals such as Aphids (Fig 8) with their cortege of companions such as ants (*Monomorium* sp) or predators such as *Chilopodae* larva. Such colonisation was found in 3 out of 4 well-established cavities on 5-month-old seedlings.

Then, colonisation by an undetermined fungus was revealed one month after by the presence of mycelium spots (yellow or white spots). As time went by, more and more cavities showed such contamination by spots or large mycelium patches (Fig 9). This fungus development was probably promoted by the presence of aphids with their sweet secretions too. After 10 months in the nursery, more than 70% of well established cavities were contaminated.

This proved that from a very early stage of seedling development, the cavity displayed all the prerequisite qualities mentioned by Breton *et al* (2005a, 2005b) to be a perfect culture chamber for *Ganoderma boninense* (SLS): hard substrate, dark environment, humidity and low competition.

This repeated degradation of the tissues around the root bases as a possible explanation of the cavity expansion could be the weakness point of the bole protection if the stroma-like structure is well developed on a significant part of the cavity wall.

Under other conditions and on other continents, such contamination by tiny animals, bacteria or whatever else could be a vector for oil palm disease.

Making the cavity beneficial for the plant

Attempts to control BSR through fungicide application or stem injection have been largely documented in the literature. These methods are summarized by Turner & Gillbanks (2003) or Corley & Tinker (2003).

More recently, use of antagonistic micro-organisms such as *Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens* or arbuscular Mycorrhizal fungi was promoted as an efficient tool to control BSR development (Karthikeyan *et al*, 2006; Jayanthi Nagappan *et al*, 2009; Mhod Ahdly Arbain and Tey Chin Chong, 2009; Nor Sarashimatun and Tey, 2009; Shamala Sundram *et al*, 2008; Susanto *et al*, 2005).

Our observations confirm that SSCs, which are always in central position, are a good target for mitigation of Basal Stem Rot (BSR) development through the application of antagonistic micro-organisms / fungi or long-lasting fungicides (Breton *et al*, 2009 a, Breton *et al*, 2009 b).

Combining efficient fungicides and / or antagonistic micro-organisms / fungi as mentioned above and the characteristics of star-shaped cavity development, it could be possible to promote more sustainable BSR control by precise application in or around SSCs.

Thus, several targets could be investigated: germinated seeds, young seedlings during transfer phases to the nursery and / or to the field, young palms or still healthy palms, palms already affected by BSR.

Germinated seeds

Antagonistic organisms could be inoculated on the germ just before transfer to the prenursery (Target 1 in Fig 10).

Young seedlings

It is possible to imagine application of antagonistic organisms or long lasting fungicide precisely located with an appropriate tool just under the seedling bole (Target 2 in Fig 11). Such an operation could be carried out during transfer to the nursery or transfer to the field.

Healthy palms

Pressurized Injection of protective / antagonistic organisms / fungi or fungicides into the star-shaped cavity could be carried out preventing SLS development (Target 3 in Fig 12). Two ways could be used to reach the SSC: through the root system, that does not injure the palm (Way 1), or via the stem (Way 2).

Affected High Conservative Value palms

After cleaning rotten tissues that generally extended well beyond the SSC, pressurized injection of curative / protective fungicide could be carried out in healthy tissues in order to protect them against BSR extension. At the same time, mounding is carried out promoting new roots emission (Target 4 in Fig 13).

PT Socfindo and CIRAD are still developing such techniques which are particularly interesting for protecting High Conservation Value palms such as outstanding parents in seed or parental gardens.

CONCLUSION

The star-shaped cavity already identified as a key factor in Basal Stem Rot development in oil palm occurs naturally during the very early stage of oil palm cultivation. According to the observations made, the first sign could be observed less than 5 months in the nursery. After 10 months nearly 95% of the seedlings bore a well-established cavity.

It was proven through the same observation that SSCs were colonised very early too by various fungi and tiny pests such as aphids opening the door for possible contamination if such fungi or tiny pests are vectors of oil palm disease.

However, advantage could be taken of SSCs for BSR control. Developing the use of long-lasting fungicide / antagonistic micro-organisms or fungi through precise application or injection into or around the SSC should offer real possibilities for sustainable ICM alongside traditional phytosanitary field practices and breeding for tolerance, which are the best long term ways of controlling BSR (Corley and Tinker, 2003).

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Table 1: Date of dissection and age of seedlings (in months after transfer to the main nursery)

Dissection	Date	Age
First	20/04/2009	1.2
Second	05/05/2009	1.7
Third	13/06/2009	3.0
Fourth	24/08/2009	5.4
Fifth	06/10/2009	6.8
Sixth	29/10/2009	7.6
Seventh	25/11/2009	8.5
Eighth	27/01/2010	10.6

Table 2: Number of roots

Date of observation	20/04/2009	05/05/2009	13/06/2009	24/08/2009	06/10/2009	29/10/2009	25/11/2009	27/01/2010
Total number of roots	10.1 ± 0.52	11.0 ± 0.73	13.9 ± 0.77	20.5 ± 1.62	23.9 ± 1.63	29.1 ± 1.57	33.0 ± 2.56	39.1 ± 3.68
New roots	2.0 ± 0.40	2.1 ± 0.58	3.4 ± 0.54	3.0 ± 0.73	3.8 ± 0.88	3.9 ± 0.60	5.0 ± 1.00	4.5 ± 1.31
Normal roots	1.7 ± 0.61	2.8 ± 0.63	5.5 ± 0.89	13.2 ± 1.35	17.7 ± 1.53	21.8 ± 1.94	25.7 ± 2.24	33.0 ± 2.31
Injured roots	6.2 ± 0.50	5.6 ± 0.41	4.1 ± 0.52	3.8 ± 0.94	1.4 ± 0.67	1.9 ± 0.70	1.6 ± 0.64	1.2 ± 0.54
Rotten roots	0.3 ± 0.24	0.6 ± 0.30	0.9 ± 0.47	0.5 ± 0.30	1.1 ± 0.52	1.6 ± 0.75	0.8 ± 0.69	0.5 ± 0.30

Table 3: Number of root-bole interfaces showing a scar or a cavity

Date of observation	20/04/2009	05/05/2009	13/06/2009	24/08/2009	06/10/2009	29/10/2009	25/11/2009	27/01/2010
No scar / cavity	20	12	13	5	5	3	0	0
Suspicious scar	0	8	7	4	1	0	0	0
Cavity	0	0	0	11	14	17	20	20
Total	20	20	20	20	20	20	20	20

Table 4: Morphology of scar/cavity on root-bole interface

Date of observation	20/04/2009	05/05/2009	13/06/2009	24/08/2009	06/10/2009	29/10/2009	25/11/2009	27/01/2010
Suspicious	0	8	7	0	0	0	0	0
Very early	0	0	0	16	13	2	2	1
S2	0	0	0	4	1	5	5	6
S3	0	0	0	0	0	9	9	8
S4	0	0	0	0	0	1	4	4
S5	0	0	0	0	0	0	0	1

Table 5: Size of scar/cavity on the root-bole interface

[illegible]

Fig. 1: Macroscopic observation of normal root – bole interface on 5-month-old seedling



Fig. 2: Rotten tissue around a root insertion on the root – bole interface



Fig. 3: Very early scar foreshadowing future star-shaped cavity



Fig. 4: Early scar developing through a root base



Fig. 5: Young SSC developing through the root – bole interface



Fig. 6: Rotten tissues extending SSC



Fig. 7: Rotten tissues extending SSC – vertical section



Fig. 8: Aphids colonizing SSC



Fig. 9: SSC colonized by fungus mycelium



Fig. 10: Target of possible action on germinated seeds

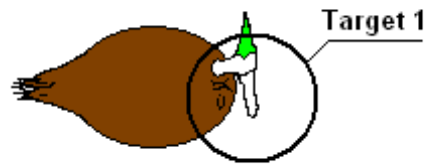


Fig. 11: Target of possible action on seedlings or young palm

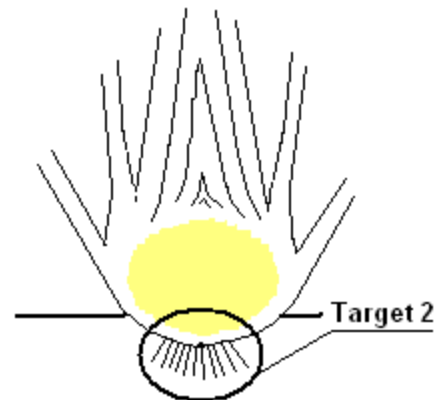


Fig. 12: Target of possible prophylactic action on adult palm

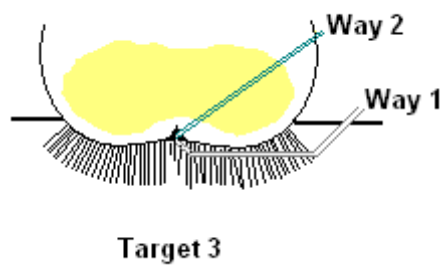
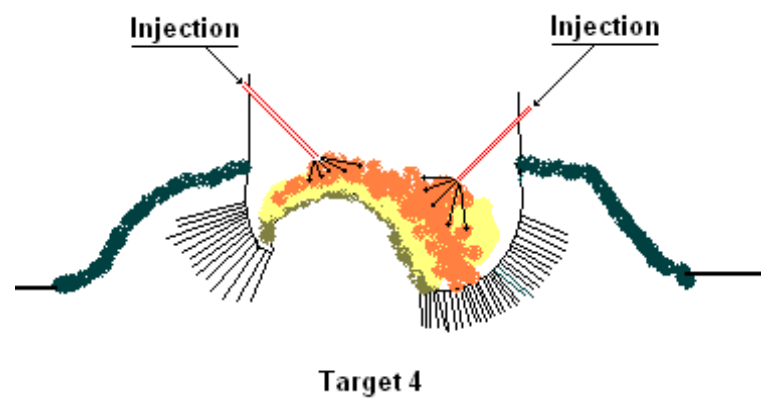


Fig. 13: target of possible curative action on adult palm



PICTURE GALLERY

S3 type of Star-shaped Cavity



S4 type of Star-shaped Cavity



S5 type of Star Shape Cavity

